Exploratory/Developmental (R21) Bioengineering Research Grants (BRG)
New Awards Fiscal Year 2004

Grant:	1R21EB003925-01	
Principal Investigator:	BURGESS, JAMES D	PHD
Title:	Tracking Cellular Cholesterol Efflux in Real Time	
Institution:	CASE WESTERN RESERVE UNIVERSITY	CLEVELAND, OH
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): This research will develop a method for tracking the cholesterol content of cell plasma membranes in real time, thus providing needed mechanistic information that cannot be obtained using traditional protocols. Specifically, the process by which, the integral membrane protein, ABCA1, mediates cholesterol efflux from macrophages (cells that become loaded with cholesterol in atherosclerosis) will be characterized. Current literature indicates that a method for evaluating the cholesterol content of the plasma membrane as a function of ABCA1-mediated cholesterol efflux is needed. The primary aim of this proposal is the further development and implementation of microelectrodes capable of oxidizing cholesterol contained in the cell plasma membrane. Platinum microelectrodes are modified with a lipid bilayer membrane containing cholesterol oxidase, an enzyme that oxidizes cholesterol. The electrode-supported lipid bilayer membrane provides two vital functions; it immobilizes the enzyme on the electrode surface in a near native environment, and extracts cholesterol from the cell plasma membrane. The platinum microelectrodes are recessed a few micrometers in the tip of a glass capillary (cavity microelectrodes) to control the distance between the cell plasma membrane and the electrode surface. Initial single cell experiments show that the enzyme-modified microelectrodes can be used to track the cholesterol content of the cell plasma membrane. Experiments are proposed to track the cholesterol content of the macrophage plasma membrane in real time during ABCA1- mediated cholesterol efflux. Experiments aimed at detecting exocytosis of HDL are also proposed. The data will be used to evaluate the validity of two proposed mechanisms that have been put forth to model the function of ABCA1 in the removal of cellular cholesterol.

Grant:	1R21EB003473-01	
Principal Investigator:	CLEEMANN, LARS	PHD
Title:	TIRF imaging of fast near-membrane Ca2+ and H+ signaling	
Institution:	GEORGETOWN UNIVERSITY	WASHINGTON, DC
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): We propose to develop total internal reflectance fluorescence (TIRF) microscopy in an imaging modality with a sharper focal plane (approximately 60 nm) and sub-millisecond time resolution (2000 frames/sec) for the study of rapid signaling near the cell membrane. To achieve this we shall use a novel optical design and operate in a mode where the response time of the fluorescent probe (e.g. Fluo- 3) is governed primarily by its diffusion out of the evanescent field (<<1 ms), not by its association time (k/off/-1 approximately- 10 ms). Preliminary experiments performed with a prototype system (approximately 100 nm focal plane, 734 frames/sec) have demonstrated proof-of-principle and resolved subsarcolammel Ca 2v signals in voltage-clamped rat atrial cardiomyocytes in terms of apparent single-channel Ca 2+ fluxes and intermittently active Ca 2+ release-components of Ca 2+ sparks. The specific aims are: -1-To sharpen the focal plane (100->60 nm) by using denser optical materials (n: 151->1.77) and purchase and use a faster, more sensitive CCD camera (734-> 2000 frames/sec). -2- To evaluate the performance of this system with respect to speed, sensitivity and noise while varying the penetration (200-60 nm) and using diffusible and non-diffusible fluorescent probes. -3-To ascertain if the subsarcolemmal Ca2+-signals in atrial cardiomyocytes can be resolved in terms of single channel Ca 2+ fluxes produced by DHP- and/or ryanodine-receptors. -4-To evaluate, in a model system of cultured adrenal chromaffin cells, if protons are co-released in sufficient numbers to modulate synaptic transmission by transient acidification of synaptic clefts. While the two listed applications are relevant to our ongoing research, we believe that implementation of the TIRF technique with sufficient sensitivity and speed to match electrical recordings of single-channel currents will be of considerable general usefulness in the study of the structures and regulatory processes that are associated with cell membranes and their immediate vicinity.

Grant:	1R21EB004353-01	
Principal Investigator:	CLEMENT, GREGORY T	PHD
Title:	Shear-mode Transcranial Ultrasound Imaging	
Institution:	BRIGHAM AND WOMEN'S HOSPITAL	BOSTON, MA
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): Ultrasound brain imaging holds the potential to provide a low cost, and portable method for imaging blood flow, detecting hemorrhaging, and diagnosing certain brain disorders. However, distortion and low signal to noise ratios (SNR) caused by the skull have severely limited the use of existing clinical devices such as trancranial Doppler sonography (TCD) and transcranial color coded sonography (TCCS). Signal degradation is caused by reflection, refraction, attenuation, and scattering by the skull. Our recent work, however, indicates that under certain conditions it is possible to propagate ultrasound through the skull with reduced distortion and higher signal amplitudes by using high incident angles. Both numeric and experimental investigation suggest this is due to the behavior of shear modes induced in the skull bone. When the ultrasound angle of entry is beyond Snell's critical angle for the longitudinal pressure wave, propagation in the bone is purely due to a shear wave. This wave then converts back to a longitudinal acoustic wave in the brain. This conversion from a longitudinal wave (skin) to a shear wave (skull) and again to a longitudinal wave (brain) does not necessarily produce a highly distorted or small-amplitude wave. Preliminary data shows that a signal obtained through the skull at high angles may be less distorted than a longitudinal one. This proposal investigates the idea that substantial improvement of transcranial ultrasound imaging can be achieved by propagating through the skull as a shear wave as opposed to a longitudinal acoustic mode. Simultaneously, a multi-cycle coded excitation sequence, devised in the present work, will significantly increase overall signal strength. The resulting images are expected to experience reduced distortion and increased an SNR, allowing clearer and more accurate brain images. The investigation will test the application of the transcranial shear mode to a number of imaging problems including the vessel detection, tumor detection, tissue morphology, and hemorrhaging in the brain. This study will provide new data on physical properties of human skulls, and will include a valuable assessment of the ability of ultrasound to detect features in the brain. The high-risk proposal could provide considerable benefits to clinical diagnostics of the brain. It could potentially offer a non-ionizing imaging method that could in operate clinically, while introducing a new technique into medical and biological imaging.

Grant:	1R21HG003448-01	
Principal Investigator:	DAVIS, RONALD W	
Title:	Single Molecule Nucleic Acid Detection with Nanopipettes	
Institution:	STANFORD UNIVERSITY	STANFORD, CA
Project Period:	2004/09/01-2006/05/31	

DESCRIPTION (provided by applicant): The long-term objective of this project is to develop a new technology that will enable rapid, single-molecule detection and identification of DNA sequences present in a biological sample. The current effort will focus on detecting nucleic acid molecules labeled with varying sizes of nanoparticles by recording changes in ionic current through a small, nanometer-scale channel in a "nanopipette." Once this detection technology has been demonstrated, the labeled oligonucleotides can be hybridized to a test sample, the unhybridized labeled molecules removed, and the remaining labeled DNA molecules can be rapidly detected on a single-molecule basis through the nanopipette. This will result in an ultra-sensitive, rapid genotyping technology that can be used for point-of-care diagnostics. The diagnostics can include the detection of pathogens or the determination of a human genotype in a clinical sample. This nanopipette DNA detection technology will also pave the way for second-generation devices, which allow higher resolution detection and could be used for rapid, single-molecule DNA sequencing, eventually realizing the possibility of sequencing an entire human genome in a matter of seconds. In this effort, Stanford will develop and demonstrate this nucleic acid detection technology with the following Specific Aims: -Nanopipette fabrication and characterization -Labeling DNA with nanoparticles -Measurement of labeled DNA

Grant:	1R21EB004324-01	
Principal Investigator:	DIMITROV, IVAN	PHD
Title:	Method for measuring ventilation-perfusion mismatches	
Institution:	UNIVERSITY OF PENNSYLVANIA	PHILADELPHIA, PA
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): Through this proposal the PI seeks to develop an advanced MRI technique to quantify regional lung perfusion-ventilation ratios, and to use this technique to further understanding of ventilationperfusion mismatches. This proposal will test the Hypothesis that the extreme sensitivity of hyperpolarized (HP) MRI can be used as a high-resolution, indirect detector for lung ventilation-perfusion (V/Q) mismatches. A secondary hypothesis is that HP-gas MRI will allow for quantification of V/Q mismatches and that the new method can be used to establish the physiological significance of sub-segmental emboli. Specific Aims: 1). Quantify the sensitivity of HP-gas MRI to indirectly detect changes in susceptibility and derive relative flow using established tracer kinetics models. 2). Measure changes in alveolar HP 3He phase and T2* maps of the lungs of healthy pigs before and after intravenous injection of gadolinium. 3). Evaluate the capabilities of HP-gas MRI for detecting segmental and sub-segmental ventilation-perfusion mismatches in the case of pulmonary emboli. Significance: Quantification of regional ventilation and perfusion is of paramount importance for the diagnosis and treatment of a variety of pulmonary disorders, including pulmonary embolus, asthma, emphysema, and interstitial lung diseases. Accurate diagnosis of V/Q mismatches requires high-resolution visualization of regional lung perfusion and ventilation. The proposed method for quantifiable V/Q MRI will be a powerful tool in understanding the complex mechanisms acting during various acute or chronic lung diseases. The results will extend our abilities to detect and diagnose lung diseases, which are the 4th leading cause of death in the US.

Grant:	1R21CA109241-01	
Principal Investigator:	DONNELLY, EDWIN F	MD
Title:	Monochromatic Phase-Contrast Breast Cancer Radiography	
Institution:	VANDERBILT UNIVERSITY	NASHVILLE, TN
Project Period:	2004/08/01-2006/07/31	

DESCRIPTION (provided by applicant): The Department of Radiology and Radiological Sciences at Vanderbilt University Medical Center has been investigating polychromatic phase-contrast radiography (PC-R) for several years. This technique has many potential uses in medical imaging as a new contrast mechanism for soft tissue imaging. Unlike laboratories utilizing a Synchroton light source (which will not be available for routine medical facilities), our technique utilizes the concept of spatial coherence, rather than temporal coherence, to obtain images strongly influenced by phase effects. The major limitation of the technique is the low tube output of the microfocal X-ray tube needed for PC-R. This project proposes to partner the investigators in Radiology who have been developing PC-R techniques with an X-ray source available at the Free Electron Laboratory (FEL) facility located on the Vanderbilt campus through a new collaboration between physicists at the FEL laboratory and the imaging researchers in the PC-R laboratory. The newly-developed X-ray source is completely independent of the Free Electron Laser itself and has been designed to function as a "table-top" source that could be generally available to medical facilities. The X-ray beam produced by this source is largely monochromatic and has an extremely high spatial coherence. In addition, it has been designed to provide a high X-ray flux. These 3arameters make it a nearly-ideal source for PC-R. The project will initially optimize the imaging parameters for monochromatic PC-R with the unique X-ray source, by investigating different geometries and energies. Once the technique has been optimized, monochromatic PC-R will be compared to absorption radiography (with both the monochromatic source and with a traditional mammography machine) on surgical breast specimens. Image quality of all images will be judged by an experienced mammographer.

Grant:	1R21EB004165-01	
Principal Investigator:	FENNELLY, GLENN J	MD
Title:	Nonpathogenic Mycobacteria:Anti-Bladder Tumor Therapy	
Institution:	YESHIVA UNIVERSITY	BRONX, NY
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): This R21 proposal is submitted in response to PA-03-058 Exploratory/Developmental Bioengineering Researcy Grants. Our overall objective is to engineer a novel anti-tumor therapy with eukaryotic expression plasmids that encode therapeutic genes delivered in nonpathogenic mycobacteria, as a safer alternative to viral gene vectors. In particular, we will study the efficacy of wild type Mycobacterium smegmatis, and Mycobacterium bovis BCG, or alternative modified mycobacteria, for the delivery of genes that express functional cytokines or co-stimulatory molecules to bladder tumor cells. Intravesicular BCG therapy, the only US FDA-approved antitumor microbial agent in the US, has contributed to a > 20% decline in death rates from bladder cancer since 1980. Intravesicular BCG augments local production of immune mediators of tumor clearance (IFN-gamma, ICAM-1 and TNF-alpha) and has direct anti-tumor activity. Nevertheless, BCG has no effect in > 20% of cases. M. smegmatis, a species that is less virulent than BCG, inhibits tumor cell growth in vitro more potently than BCG. Previous studies by us, and others, demonstrate that both S. flexneri and S. typhimurium can deliver plasmids to eukaryotic cells for genetic immunization. In a set of pilot experiments, we tested the ability of M. smegmatis to deliver eukaryotic expression plasmids to mammalian cells and clearly demonstrated, for the first time, that M. smegmatis can deliver plasmids expressing the green fluorescent protein from a eukaryotic promoter to macrophages. Using this discovery, we plan to develop M. smegmatis and BCG as safe and efficient vectors for the delivery of ICAM-1 and TNF-alpha eukaryotic expression plasmids as gene therapy against bladder carcinoma in humans. Specifically, we propose to: 1. Optimize the ability of wild type M. smegmatis and BCG to deliver Mycobacterial Mammalian Shuttle plasmids (MMSP) to macrophages and murine or human bladder tumor cells in vitro and in mice. 2. Determine whether infection of murine bladder tumor cells with recombinant Mycobacteria harboring MMSP that encode murine ICAM-1 or TNF-alpha augment tumor cell expression of functional ICAM-1 or TNF-alpha in vitro and, 3. Evaluate the ability of recombinant Mycobacteria harboring MMSP that encode ICAM-1 or TNF-alpha genes to enhance tumor regression in a murine MB49 syngeneic orthotopic bladder cancer model using C57BL/6 (immunocompetent).

Grant:	1R21DC006665-01	
Principal Investigator:	FINLEY, CHARLES C	PHD
Title:	Physio-anatomical Factors in Cochlear Implant Outcomes	
Institution:	UNIVERSITY OF NORTH CAROLINA CHAPEL HILL	CHAPEL HILL, NC
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): Because mean performance levels across different cochlear implant systems are highly similar, and large outcome variation is observed across patients with the same device, it is hypothesized that physioanatomical factors play a significant role in determining outcome in individual cochlear implant patients. The long-term objective of this application is to determine how well the peripheral factors of (1) cochlear size and shape, (2) electrode insertion depth and scale tympani position and (3) neural survival and physiological responsiveness as measured in individual implant subjects can account for the performance variation seen across the same subjects. Factors related to cochlear anatomy and electrode placement can largely be measured by high-resolution CT imaging. Peripheral physiological responsiveness can be measured grossly using intracochlear-evoked potentials (IEP), whereas there are no direct in vivo measures of neural survival. This project will seek to estimate the combined influence of physiological responsiveness and variable neural survival in an individual subject by comparing IEP measures against a reference electro-anatomical-neural computational model that assumes full neural survival and uniform neural characteristics. The electro-anatomical reference model will be based however on the cochlear anatomy and electrode placement as determined by CT imaging in each individual subject. Multiple linear regression analysis will be used to examine the relative and combined contributions of each of these factors in accounting for variance in the subject performance outcomes. The immediate objective of this exploratory project is to construct and validate the necessary tools to make these measures and then conduct a small pilot study in 3-6 Nucleus and 3-6 Clarion implant subjects, hopefully leading to a subsequent large-scale study. Specifically, this study will seek to extend the fine spatial resolution of pre- and post-implantation high-resolution CT images using advanced preprocessing techniques before back projection, followed by subvoxel image interpolation and segmentation using shape-based interpolation kernels derived from micro-CT images of cadaveric temporal bones. This fine resolution CT image voxel-space will then be converted on a voxel-by-voxel basis into a finite-element-based electro anatomical neural model. Electrical fields and IEP responses will be computed for specific stimulation conditions and compared with measured IEP responses in a pilot study.

Grant:	1R21CA108853-01	
Principal Investigator:	FREYER, JAMES P	
Title:	New Model of the Tumor Microenvironment	
Institution:	UNIVERSITY OF CALIF-LOS ALAMOS NAT LAB	LOS ALAMOS, NM
Project Period:	2004/09/17-2006/08/31	

DESCRIPTION (provided by applicant): The over-all goal of this two-year project will be to develop and refine a novel three-dimensional tumor model in which we can measure spatial distributions of microenvironmental parameters and directly compare these with alterations in cellular physiology and functional genomics. We propose three Specific Aims to develop and initially validate this new experimental model system. The first Aim is to develop a novel three dimensional cell culture chamber for spatially-resolved cell physiology, gene and protein expression. This chamber will provide steady-state gradients of microenvironmental factors that are spatially correlated with gradients in cellular physiology, metabolism and gene/protein expression. The second Aim is to develop NMR 1-D imaging methods for oxygen, pH, glucose/lactate and cell density/flow. These methods will provide 1-D maps of concentration gradients that are spatially correlated with the corresponding gradients in cellular parameters. The third Aim will be to develop and validate a mass transport model for fitting the NMR concentration profiles in order to obtain cellular metabolic parameters. The aims are highly interactive: improvements in spatial resolution of the NMR imaging methods will be coupled to development of a more tissue-like system for cell culture, and visa versa. The data from each iteration of the system will be analyzed using the mass transport model, which will, in turn, be refined based on the results obtained. This will be the first 3-D cell culture system available for measuring the effects on tumor cells of exposure to known combinations of metabolite and catabolite gradients. Although the aims of this two-year application are primarily to develop, validate and refine the new model system, we produce preliminary data demonstrating the usefulness of this system for our ongoing projects in studying the tumor microenvironment. We anticipate application of this new system to answer fundamental questions about the regulation of cellular metabolism, proliferation, physiology, and gene/protein expression under multi-component microenvironmental stress, which closely mimics the situation in tumors. This system will be useful in a wide variety of other basic and applied cancer research areas, including drug development, radiobiology, and non-invasive diagnosis. The new tumor model system should also serve as an excellent platform for improving biomedical imaging, particularly NMR microimaging. The system is also potentially very useful for applied research on cell culture systems for biomaterial production and artificial organs. Finally, this system should prove useful for investigating the regulation of metabolism and physiology in microbial systems, a current area of intense interest.

Grant:	1R21GM070929-01	
Principal Investigator:	GEDDES, CHRIS D	PHD
Title:	Metal-Enhanced Fluorescence RNA Sensing	
Institution:	UNIVERSITY OF MD BIOTECHNOLOGY INSTITUTE	BALTIMORE, MD
Project Period:	2004/08/06-2006/07/31	

DESCRIPTION (provided by applicant): Recently we have explained and developed a new fluorescence phenomenon that relies on the interactions of fluorophores with metallic particles and surfaces. These interactions can increase the quantum yield of weakly fluorescing species, provide spatially localized excitation and even improve probe photostability. Our new technology clearly demonstrates that these novel effects can result in up to a million-fold more photons per fluorophore, which when applied to assay sensing platforms will provide the equivalent of PCR or ELISA sensitivity, without any amplification steps. We subsequently envisage developing a new metallic-surface micro-assay based detection system, to serve downstream as a field deployable bio-terrorism sensor or a rapid gene profiling platform for clinical diagnostic applications. This R21 proposal is designed to demonstrate that we can indeed detect RNA targets with extremely high sensitivity and selectivity. Furthermore we believe that the likely successful outcome of high sensitivity detection in this proposal will lead to a generic platform for the development of high-sensitivity micro-assays for both RNA and DNA targets. The Metal-Enhanced Fluorescence RNA sensing assay functions as follows. Two DNA oligonucleotide probes complimentary to different regions of the target mRNA will be synthesized. One probe will be 5'-labeled with biotin, and the other with fluorescein. The DNA probes will be annealed in solution to RNA targets in a bulk RNA population under reaction conditions optimized for each probe pair. The annealing mixture will then be applied to a streptavidin-coated silver surface at the same temperature, permitting capture of specific RNA:DNA hybrids through binding of the biotin moiety. After extensive washing, the amount of bound RNA will be quantified by Metal-Enhanced Fluorescence of the retained fluorescein-coupled DNA probe. We intend to determine the sensitivity of the assay by using series dilution of the RNA and subsequently determine and compare the detection levels using both 1 and 2-photon excitation of fluorescein. In addition we aim to demonstrate the enhanced photostability of fluorescein on the silver assay.

Grant:	1R21HL076457-01	
Principal Investigator:	GNATENKO, DMITRI V	PHD
Title:	Platelet transcriptome analysis from small blood volumes	
Institution:	STATE UNIVERSITY NEW YORK STONY BROOK	STONY BROOK, NY
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): Human blood platelets play critical roles in normal hemostatic processes and pathologic conditions such as thrombosis, vascular remodeling, inflammation, and wound repair. Generated as cytoplasmic buds from precursor bone marrow megakaryocytes, platelets are enucleate and lack nuclear DNA, although they retain megakaryocyte-derived mRNAs. Towards the goal of defining the molecular anatomy of the platelet transcriptome, we have adapted complementary techniques of microarray and serial analysis of gene expression (SAGE) for genetic profiling of highly purified human blood platelets (Blood 101:2285-2293), and demonstrated the potential applicability of this approach for the molecular analysis of a rare platelet disorder (essential thrombocythemia). While these observations established the initial proof-of-principle supporting this research direction, current platelet isolation procedures require plateletpheresis and relatively cumbersome purification methods for optimal determinations, limiting wider applicability. During the tenure of this grant, we propose to develop a miniaturized system for high-throughput platelet transcriptome analysis. In specific aim 1, we will adapt and develop mechanical shear as an efficient methodology for separation of ultra-pure platelets from whole blood (1 mL), followed by mRNA isolation and representative transcript amplification uniquely adapted for small RNA yields. In specific aim 2, we will develop a customized platelet cDNA chip for confirmatory studies of amplified platelet mRNA fidelity as established by microarray analysis. If successful, this project will develop the appropriate infrastructure and methodologies for more comprehensive profiling of larger data sets, a major long-term goal of this area of investigation. Given the importance of platelets in cardiovascular disease and stroke, these studies will have considerable implications for novel gene discovery, and for molecular diagnostics targeted at large patient populations.

Grant:	1R21EB003516-01	
Principal Investigator:	HASELTON, FREDERICK R	
Title:	Lagrangian detection of biomolecular interactions	
Institution:	VANDERBILT UNIVERSITY	NASHVILLE, TN
Project Period:	2004/05/01-2006/04/30	

DESCRIPTION (provided by applicant): Patterns of gene expression and protein profiles are currently revolutionizing the understanding of biology and disease and are likely to become diagnostic and prognostic tools. Once these patterns are established, methods to implement them will be required. We have designed a platform which rapidly matches the characteristics of an unknown sample with established expression or protein profiles. The approach is characterized by computer controlled testing for pattern features with adaptive feedback to increase sensitivity and reliability. It will have greatest utility for identifying established patterns described in terms of the presence or concentration of 100s to 1000s of key molecular structures. Current high-throughput methods for profiling of an unknown sample, such as DNA microarrays for gene expression profiling, can be characterized as Eulerian design - known capture probes are fixed in space by attachment to a solid substrate and hybridized by target diffusion. Biorecognition is inefficient querying only a fraction of the target solution, target-probe interactions occur slowly, and target-probe binding occurs under uniform conditions. This application describes an alternative Lagrangian design - probes are arranged on a filament and moved through nanoliter reaction zones for biorecognition testing, processing, and analysis. The extremely small dimensions enable complete, rapid, and complete target-probe interactions. The flexible and adaptive features permit tailoring reaction conditions to each capture probe-target interaction. We have identified several key components that are critical to the success of this new platform technology. These are the identification of an appropriate fiber, deposition of probe, development of the small volume reaction compartment with immiscible surface tension valves, and a specialized apparatus for computer controlled fiber transport through multiple reaction chambers. Although we have limited experimental data, the data we do have, suggests that each of the key components of the proposed system is feasible. This technology will provide a new tool for the identification of patterns present in gene expression or protein profiles enabling these profiles to be fully utilized for diagnostic and prognostic purposes.

Grant:	1R21HL076241-01	
Principal Investigator:	HOH, JAN H	PHD
Title:	Micromechanical Characterization of Endothelial Cortex	
Institution:	JOHNS HOPKINS UNIVERSITY	BALTIMORE, MD
Project Period:	2004/04/01-2006/03/31	

The long-term goal of the proposed research is to understand the relationship between cell mechanics and barrier functions of the vascular endothelium. Changes in the permeability of endothelia are important in processes such as angiogenesis, arthrosclerosis and inflammation. The cytoskeleton of vascular endothelium is known to be highly dynamic and is thought to be the primary determinant of cellular mechanics. Further, changes in the cytoskeleton have been shown to directly affect the endothelial barrier. The hypothesis put forth here is that such cytoskeletal reorganization results in a remodeling of the local mechanical properties, which in turn will have important consequences for barrier function. Thus, we propose an effort to characterize the local mechanical architecture of the cortex of a model endothelial cell (Bovine Pulmonary Artery Cells), and study the mechanical remodeling of these cells. To achieve this goal we here propose a pilot project to develop the imaging methods necessary to visualize cortical mechanics and to establish a clear connection between the cytoskeleton and cortical mechano-architecture. This pilot project has two specific aims. First we will use atomic force microscopy to produce high-resolution images of the local mechanical architecture of the cortex in living endothelial cells. These images will reveal how the cortex of these cells is organized, and how it remodels with time. We will also characterize the local mechanics through indentation measurements, and relate these measurements to the cortical morphology. Second, we will determine the molecular components that determine the mechanical architecture of vascular endothelial cells by using biochemical labeling and pharmacological approaches. Correlated imaging between confocal microscopy of cells labeled with antibodies specific for actin, tubulin and other cytoskeletal components will be used to identify what cellular structures are responsible for the mechanical features seen in the AFM images. In addition, pharmacological agents will be used to disrupt specific structures, in particular cytoskeletal structures, and correlate that to loss of mechanical features. The successful completion of this project provides the necessary technology and scientific foundation for a complete study of cortical mechanics of the vascular endothelium, and mechanical remodeling in normal and diseased cells.

Grant:	1R21EB004658-01	
Principal Investigator:	ILANGOVAN, GOVINDASAMY	PHD
Title:	Microxymetry Techniques for Cardiomyocyte Respiration	
Institution:	OHIO STATE UNIVERSITY	COLUMBUS, OH
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): Measurements of oxygen consumption and generation of reactive oxygen species (ROS) in cardiomyocytes, assume importance in pathophysiology of ischemic heart disease because large quantities of oxygen free radicals are generated during ischemia/reperfusion, and the produced free radicals impair the heart function. Though the oxygen consumption and free radicals generation occur simultaneously, till date there is no method known to simultaneously measure both oxygen consumption and free radicals generation in a single experiment. The purpose of the present grant proposal is to explore the application of a new EPR technique called "Microxymetry" to achieve this goal, i.e., the real time quantitation of both the oxygen consumption and free radicals generation, in a single experiment. The present approach combines the EPR oxymetry and spin trapping into one, to get both oxygen concentration and free radicals concentration in one step. The specific aims of the proposal are: (i) to study the respiration (both as such and stimulated by added stimulants) of cardiomyocytes (both cultured cells and freshly isolated from perfused hearts) at different conditions. Particularly, in these systems, how the simulated ischemia alters oxygen consumption rate of cardiomyocytes will be studied in detail. The ROS generated during the respiration of these ceils at physiologically altered conditions, will be studied simultaneously and the relationship between oxygen consumption and the amount of ROS formed will be evaluated at various deleterious conditions like hypoxia. Further, the effects of simulated ischemic preconditioning and induction of heat shock proteins on the oxygen consumption rate and ROS production will also be studied using the proposed microxymetry; (ii) to study the respiration of isolated heart mitochondria using the proposed microxymtery. Similar to the cardiomyocytes, the heart mitochondria will be subjected to different conditions like hypoxia and normoxiaI PC cycles and the effect of these treatments on mitochondria respiration will be studied. It is also proposed to use this technique to study the specific inhibitors on complex I and complex Ill and their effect on the state 3 and state 4 respirations and the subsequent influences on the oxygen consumption of mitochondria. Overall, by establishing this new technique, it is possible to simultaneously measure the oxygen consumption and ROS production in microliter volumes of cellular and sub-cellular components.

Grant:	1R21AR051514-01	
Principal Investigator:	KANG, JAMES D	MD
Title:	TIMP Gene Transfer to Alter Course of Disc Degeneration	
Institution:	UNIVERSITY OF PITTSBURGH AT PITTSBURGH	PITTSBURGH, PA
Project Period:	2004/06/04-2006/05/31	

DESCRIPTION (provided by applicant): The prevalence of musculoskeletal impairments of the spine in the U.S. is estimated to be greater than 18 million--many of which are the direct or indirect result of intervertebral disc degeneration (IDD). The limited available technology for the treatment of the pathologic and disabling conditions arising from IDD generally is highly invasive (e.g., surgical discectomy and fusion), manifesting a certain degree of complications and unsatisfactory clinical outcomes. To date, little effort has been made to directly treat the underlying problem of disc degeneration--a chronic process characterized in part by progressive loss of proteoglycans leading to disc dehydration, alterations in disc structure, and impaired disc function. Recent advancements in molecular biology have made it possible to contemplate treating the intervertebral disc itself at a molecular level to prevent or delay the progression of IDD. We hypothesize that imbalance in the synthesis and catabolism of certain critical extracellular matrix (ECM) components (a final common pathway in IDD) can be mitigated by the transfer of genes to intervertebral disc cells encoding factors that modulate synthesis and catabolism of these ECM components--thereby improving disc composition, structure, and ultimately function. The proposed study will test the efficacy of adenovirus-mediated delivery of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) genes in interrupting the degenerative cascade in a novel rabbit model of disc degeneration, using functional outcomes and MRI to assess preservation of disc composition, structure, and function. The significance of this study includes an improved basic science understanding of the pathogenesis and pathophysiology of IDD, as well as the identification of novel therapeutic approaches to the clinical treatment of IDD.

Grant:	1R21DC006466-01	
Principal Investigator:	KUO, ARTHUR D	PHD VISUAL PSYCHOPHYSICS
Title:	Control of Balance during Human Walking	
Institution:	UNIVERSITY OF MICHIGAN AT ANN ARBOR	ANN ARBOR, MI
Project Period:	2004/01/15-2005/12/31	

DESCRIPTION (provided by applicant): The long-term goal of this research is to understand how humans continuously stabilize their bodies while walking. Balance is necessary to prevent falling, an issue relevant to the rehabilitation of individuals with vestibular and other sensory impairments, as well as elderly adults who suffer from decreased sensory function. Although a great deal is known about postural balance while standing, less is known about balance while walking, because translation of the body makes it more difficult to make measurements and apply perturbations. We will develop a new experimental device that will make it possible to manipulate balance during walking, so that the resulting human control responses can be measured. We will then use this device to study common gait adaptations such as to step length and step width, and their impact on stability. These adaptations will be studied in both young and elderly subjects. The Specific Aims of this project are: 1. To design and fabricate an active device that can externally stabilize or de-stabilize the body during treadmill walking, for use in testing and assessing control of balance. This device will provide a means to experimentally manipulate the body's degree of stability, or apply small perturbations during walking. 2. To determine the relationship between the degree of body instability and characteristics of walking such as step length and width, step variability, and metabolic cost. We will test the hypothesis that these characteristics are interrelated due to the need to control balance by adjusting gait parameters and foot placement. 3. To perform a system identification of the human control system that stabilizes balance during walking. We will use the external stabilization/de-stabilization device to apply small noise-like perturbations to subjects walking on a treadmill, measure their associated control responses, and identify the systematic feedback responses that are used to maintain continuous stability during walking.

Grant:	1R21HL079419-01	
Principal Investigator:	LI, SONG	PHD
Title:	Proteomic Profiling of Stem Cell Differentiation	
Institution:	UNIVERSITY OF CALIFORNIA BERKELEY	BERKELEY, CA
Project Period:	2004/05/01-2006/04/30	

DESCRIPTION (provided by applicant): Bone marrow mesenchymal stem cell (BMMSC) has potential to differentiate into different cell types. The long-term goal of this research is to control BMMSC differentiation in vitro and use BMMSC-derived smooth muscle cell (SMC) to construct tissue-engineered vascular grafts. The cyclic mechanical strain in the vessel wall and transforming growth factor 13(TGF-B) play important roles in SMC differentiation and vascular remodeling. The investigator has shown that both mechanical strain and TGF-I31 increase the expression of SMC markers in BMMSC. However, the underlying mechanisms and the proteomic changes of BMMSCs during differentiation are not well understood. Proteomic profiling provides a systematic and powerful approach to studying stem cell functions. The investigator hypothesizes: (1) BMMSC and its differentiation can be characterized by the expression of specific protein markers, and (2) mechanical strain and TGF-B] regulate BMMSC differentiation through distinct mechanisms and synergize the differentiation of BMMSCs into SMCs. Two specific aims are proposed in this exploratory/development study. In Aim 1, the investigator will determine the proteomic profile of BMMSC and identify potential markers of BMMSC. TGF-B] and other differentiation factors will be used to induce the proteome changes and differentiation of BMMSC. A comprehensive strategy using state-of-the-art technologies will be employed for proteome analysis. 2D gel electrophoresis and multidimensional liquid chromatography will be used for protein/peptide separation. Mass spectrometry will be used for protein identification and characterization. A reference map of BMMSC will be generated. The proteins only expressed in undifferentiated BMMSC will be identified. In Aim 2, the investigator will determine the proteome changes in BMMSC in response to mechanical strain and TGF-B. The proteins with changes in expression and post-translational modifications will be identified. Immobilized metal affinity capture will be employed for phosphopeptide enrichment. The global mechano-chemical signal transduction during BMMSC differentiation will be determined, and a map will be generated for the signaling pathways differentially regulated, shared, and synergized by mechanical strain and TGF-B]. Categorization and cluster analysis will be performed to correlate the proteins and signaling pathways. This study will advance our knowledge on BMMSC proteome and differentiation, and lead to more focused and in-depth biological studies in the future. The successful accomplishment of the goals will have high impact on stem cell engineering, and provide a rational basis for engineering BMMSC for vascular tissue repair.

Grant:	1R21CA108677-01	
Principal Investigator:	LOW, DANIEL A	PHD
Title:	microRT	
Institution:	WASHINGTON UNIVERSITY	ST LOUIS, MO
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): The overall aim of this project is to begin development of a novel conformal small-animal irradiator, suitable for studying genetic markers of radiation response and radiobiological models at therapeutic dose levels. There has been a revolution in functional imaging that has coincided with the development of small animal imaging devices, including microCT, microMR, and microSPECT. Many of the institutions that have acquired these devices use them to study the efficacy of molecular markers for the diagnosis, detection, and characterization of cancer. One important component of these studies is the evaluation of response to therapeutic doses of radiation; however, the technology of animal irradiation is crude, making reproducible irradiation of in situ tumors and normal organs difficult. We propose to develop a small animal irradiator (microRT) that delivers customized, conformal dose distributions to rats and mice that are accurately localized, with accurate dosimetry, and conformal therapy treatment planning, to support quantitative molecular imaging studies and radiobiological experiments. The system will be based on high activity, small volume, 192Ir radiation sources, and the radiation delivery will be computer controlled and automated. Our first aim is to design a prototype small-animal irradiator based on an 192Ir source, according to the specifications defined by a multidisciplinary radiobiology and animal imaging research team. Our second aim is to build a prototype irradiator using the source and collimator design developed in SA1. The third aim is to validate that the prototype built in SA2 meets the specifications developed in SA1. The successful completion of these specific aims will open a new field of preclinical micro imaging-guided radiobiology investigations on small animals.

Grant:	1R21AR051048-01	
Principal Investigator:	MAJUMDAR, SHARMILA	PHD OTHER AREAS
Title:	SPECTROSCOPIC MARKERS OF DISC DEGENERATION	
Institution:	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	SAN FRANCISCO, CA
Project Period:	2004/08/23-2006/06/30	

DESCRIPTION (provided by applicant): Degeneration of the intervertebral disc is a common ailment in working-age adults, affecting between 65% and 80% of the population. The intervertebral disc is thought to be a source of pain in these individuals and disc pathophysiology is well studied. The most consistent chemical change observed with degeneration is loss of proteoglycan and associated loss of water. Magnetic resonance (MR) imaging methods have been used to study disc degeneration by observing the changes in water content, relaxation and diffusion, which are an indirect by-product of alterations in biochemistry and proteoglycan content. The underlying hypothesis of this proposal is that spectroscopic markers of disc degeneration such as proteoglycan changes are detectable using high resolution Magic Angle Spinning (HRMAS) and reflect biomechanical and biochemical changes related to disc degeneration. After the milestones of this exploratory project are established it is anticipated that in vivo proton spectroscopy methods for studying these metabolites may be developed and may provide a means for in situ disc biochemical characterization and provide important, but currently unavailable information for the clinical management of low back pain. The specific goals of this project are to characterize the disc metabolites, their relative ratios and relaxation properties and to identify spectral markers for degeneration using HRMAS. The marker characteristics will be assessed as a function of Thompson Grade (degeneration) and site (annulus vs. nucleus). In addition the correlation between these markers and the biochemical composition as determined by proteoglycan and collagen assays will be established. Whole disc biomechanical properties will be characterized using three material coefficients: effective permeability of the vertebral endplate, strain dependence of the nuclear swelling pressure, and the viscoelastic behavior of the annulus fibrosus and their association with the spectral markers will be established.

Grant:	1R21EB003403-01	
Principal Investigator:	MANALIS, SCOTT R	PHD
Title:	Suspended microchannels for biomolecular detection	
Institution:	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	CAMBRIDGE, MA
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): Proteins and their byproducts play a critical role in nearly every event that takes place within living cells. The understanding of many of these events is furthered by the ability to profile the concentration of specific proteins as a function of time and various physiological conditions. However, the rate at which these parameters are measured by current methodology is often limited by requirements for large sample volumes and time-consuming modifications of the molecules under investigation. Here we address these limitations by proposing a detection system based on microfluidic channels whereby the channels themselves are the detectors. The key innovation is that the mechanical properties of a suspended microfluidic channel with a thickness near a micron will allow protein concentration to be determined by measuring a mass-dependent resonant frequency of the microchannel. The suspended channel mass is altered either by proteins that enter into the channel volume, or by specific binding of proteins to capture molecules on the channel walls. Successful development of the suspended microchannel detector will result in a real-time and labelfree method that will provide an alternative to fluorescence for protein detection. Specifically, suspended microchannel detectors will offer two advances. First, is scalability: ultimately 10[2]-10[3] detectors could be operated within a square centimeter with a volume of less than 100 pL per detector. Second, we estimate that the suspended microchannel will achieve a detection limit in the range of 10[-18] is to 10[-19] g/mu m 2. This limit, which corresponds to less than 10 proteins/mu m 2, is nearly 100-fold more sensitive than other label-free methods such as the quartz crystal microbalance. We anticipate that an array of suspended microchannel detectors will substantially increase the throughput and utility of protein assays that are used for furthering the predictive power of system-level modeling. The suspended microchannels will also be useful for enabling point-of-care medical diagnostics.

Grant:	1R21NS048270-01	
Principal Investigator:	MEANEY, DAVID F	PHD
Title:	A new technology for engineering axonal growth	
Institution:	UNIVERSITY OF PENNSYLVANIA	PHILADELPHIA, PA
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): Regenerating axons within the central nervous system (CNS) remains a fundamental challenge in neuroscience. Recently, we have shown that a large number (10/5) of axons integrated with CNS neuronal cultures will grow rapidly (8-10 mm/day) and over long distances (>5 cm) if the axons are placed under a continuous mechanical tension. We feel the impact of this discovery could be significant. This technique provides a method to culture cell transplants for bridging lesions in the white matter that are centimeters long, distances that are not readily traversed with such a large number of axons using other techniques (e.g., ensheathing cell transplants, directed material scaffolds, controlled release). In addition, this model represents an opportunity to study the mechanisms of accelerated axonal growth in a large population of axons that was previously not possible. However, the technology is at a critical nascent stage with risk - it is not widely used or available to investigators, and we do not know if axonal tracts developed with this technique have viable electrophysiological function. In this proposed, we will build the appropriate technical infrastructure for rapidly culturing a large number of cell transplant constructs using commercially available materials, creating a more generalizable resource for the neuroscience community. Embedded within this re-design of the system is to allow for the measurement of electrophysiological properties of the constructs. Once developed, we use this to propose a series of studies on how a specific cytoskeletal component (neurofilaments) may be a key limiting factor in controlling the growth rate with this technique.

Grant:	1R21DE016348-01	
Principal Investigator:	MITCHELL, JOHN C	PHD
Title:	Biomimetic Adhesive for Mineralized Tissues	
Institution:	OREGON HEALTH & SCIENCE UNIVERSITY	PORTLAND, OR
Project Period:	2004/09/28-2006/08/31	

DESCRIPTION: Dental adhesives have major limitations, including: inadequate bond strength to dentin to overcome forces generated by contraction of the composite restorative; gap formation that leads to bacterial penetration and staining at the margins with the tooth surface; and limited durability due to polymer degradation and a limitation of the joint from water sorption and stressing during clinical service. These limitations create problems for the patient, including post-operative tooth pain, deterioration of the health of the tooth, and the need for costly re-treatment. This project seeks to develop materials that can produce reliable and durable bonds to tooth surfaces. This material will be capable of "self-healing" the interfacial porosity that forms immediately after placement and during clinical service, due to the development of a biomimetic approach. In addition, this material is expected to be useful as an adhesive for all hard tissues in the body. This work has two long-range goals: 1) Prepare novel, hard tissue adhesive biomaterials with the capacity for self-healing; and 2) identify the mechanism of adhesion and sealing through physical and chemical characterization of the interface between this biomaterial and hard tissues. The main objective is to create a biomimetic process to: 1) Eliminate the formation of nano- and micro-scale leakage at the tooth/adhesive interface; and 2) improve the bond strength between a Dental adhesive material and tooth structure. We will prepare a series of bioactive sol gel glass-containing Dental adhesives, and assess their physical, chemical, and mechanical properties, identifying those compositional factors, structural properties, and placement methods that will improve the seal and bond between the Dental adhesive and the tooth. Additionally we will characterize the changes in the interface between tooth and restoration.

Grant:	1R21EB003452-01	
Principal Investigator:	OKAMURA, ALLISON M	PHD
Title:	Biomechanical Modeling for Steerable Needles	
Institution:	JOHNS HOPKINS UNIVERSITY	BALTIMORE, MD
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): The long term goal of this work is to harness the effect of bevel tip needle bending to provide accurate, dexterous targeting for percutaneous therapies. New results in needle and tissue modeling, combined with robot motion modeling, will facilitate new models, hardware, control, and planning techniques for steering flexible needles inside soft tissue. Using path planning, control, and kinematics results from the field of robotics, we plan to demonstrate that an appropriately designed needle can be steered through tissue to reach a specified 3-D target. Even more compelling is that the methods we propose will allow needles to be steered to previously inaccessible locations in the body, enabling new minimally invasive procedures. The first step in needle control is to obtain an accurate model. Thus, the specific goals of this exploratory/developmental application are to under stand and model the biomechanics of needle motion. This is a high-risk activity because there has been no previous work to successfully model the 3-D path of flexible needles through soft tissue. The work is high impact because accurate 3-D needle modeling can be used to (1) improve targeting, thus enhancing the performance of many percutaneous therapies and diagnostic methods, (2) plan needle paths that steer around obstacles, and (3) develop realistic simulators for physician training and patient-specific planning. Working closely with physician collaborators, the investigators will study needle insertion scenarios that are relevant to improving the quality of health care. The specific aims are as follows: (1) Develop and validate a deterministic biomechanical model of needle insertion through un deformed tissue. The bending of the need le tip will be a function of tissue properties, needle properties and input parameters. Refine and test the model on phantom tissues. Select needle design parameters that facilitate steering. (2) Further refine the model of Aim 1 to include the effects of constraint and input uncertainties, generating a stochastic biomechanical model of needle insertion through undeformed tissue. Compute the space of possible needle tip positions for a given set of inputs and determine the probability of acquiring specific targets. (3) Further refine the models of Aims 1 and 2 to include the effects of both tissue deformation and inhomogeneous tissue properties. A particular challenge is the development of new modeling techniques that simultaneously handle needle and tissue deformation.

Grant:	1R21EB004514-01	
Principal Investigator:	PEATTIE, ROBERT A	PHD
Title:	Crosslinkable Hydrogels for Growth Factor Release	
Institution:	OREGON STATE UNIVERSITY	CORVALLIS, OR
Project Period:	2004/09/30-2006/08/31	

DESCRIPTION (provided by applicant): The goal of the proposed project is to develop and evaluate novel hyaluronic acid (HA)-based hydrogel films optimized for controlled release of desired peptide growth factors (GFs) while simultaneously providing an advantageous environment for pre-seeded cell growth. Synthetic extracellular matrices (sECMs) fabricated from such biopolymer networks can serve as the foundation for tissue and organ growth both in vitro and in vivo. However, at present the clinical utility of most synthetic matrices is limited by poor regulation of the specific coordinated sequences of GF releases that typically drives tissue maturation in vivo. In the proposed project, an sECM containing small amounts of heparin (Hp), which has been shown to substantially improve control of the time course of release of sequestered GFs, will be fabricated from chemically modified HA, or HA and gelatin (Gtn). Conjugate addition chemistry will be employed to crosslink mixtures of HA-DTPH, Gtn-DTPH and Hp-DTPH, using polyethylene glycol diacrylate (PEGDA) as crosslinking agent. Vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) will be added to the HA-DTPH solution non-covalently prior to crosslinking. The relation between Hp concentration and rate of in vitro GF release from these matrices will be investigated by ELISA. Subsequently, gel samples fabricated with a minimal amount of Hp consistent with sustained GF release will be implanted in a series of mouse ear pinnas, and the angiogenic activity of released GF in vivo quantified by measurement of elicited new microvessel growth. In addition, the potential for controlling tissue phenotypic response at the gene expression level with these imlants will be studied in the same mouse model. Tissue samples will be retrieved at a series of time points post implant, and expression changes due to controlled growth factor release determined by microarray and RT-PCR analysis.

Grant:	1R21EB004483-01	
Principal Investigator:	PICHLER, BERND J	PHD
Title:	An Integrated PET-MRI System for Molecular Imaging	
Institution:	UNIVERSITY OF CALIFORNIA DAVIS	DAVIS, CA
Project Period:	2004/09/23-2006/08/31	

DESCRIPTION (provided by applicant): An Inteqrated PET-MRI System for Molecular Imaqinq Non invasive molecular imaging in small animals is an emerging field of research. Especially positron emission tomography (PET) reveals information about ongoing functional processes in living organism, such as metabolism or even gene expression. Its importance, especially in basic cancer research is tremendous. Although PET is nearly unbeatable in sensitivity (nano molar), its drawback is clearly the lack of anatomical information. Even a spatial resolution of about I mm can not provide sufficient anatomical structure. The combination of PET with another imaging device which reveals detailed morphological information would be a huge advantage and highly beneficial for molecular imaging. The modalities, providing best anatomical information are x-ray computed tomography (CT) and magnetic resonance imaging (MRI). Whereas CT requires a high radiation dose to achieve sufficient resolution, which might change biological function in the animals and potentially the entire animal model, MRI seems to be harmless. Most importantly, MRI provides a much better soft tissue contrast than CT, even without any contrast agents. The goal of this proposal is the development of an integrated PET-MRI system for simultaneous data acquisition in motecular imaging of small animals. Our latest PET detector development, in collaboration with Concorde Microsystems, Knoxville, TN, is the cornerstone for this work: a 10x10 LSO block detector (crystal size 2x2x10 mm 3) read-out by compact monolithic 3x3 APD arrays. The 10xl multiplexing minimizes the required electronics. The compact LSO-APD detectors can be arranged as an insert between RF-coil and gradient set in a 7 Tesla MR scanner, allowing simultaneous PET and MR scanning of mice. The encouraging results achieved with the LSO-APD detectors and dedicated integrated readout electronics, in combination with preliminary results from tests of different materials, such as the LSO-APD PET detectors, plastics, and electronic boards in the 7 Tesla field indicate the feasibility of this project. However, if one part of the mosaic does not allow a simultaneous data acquisition, several fall-back-solutions are worked out, to make at least a sequential PET-MR imaging possible, providing morphological and functional information with highest spatial accuracy, which could never be achieved with two separate systems. In addition, the future applications of such a combined system go far beyond the work proposed in this grant. A combined PET-MRI providing also magnetic resonance spectroscopy information or a PET data correction (attenuation or partial volume effect) based on the MR image would be very valuable and provides a lot of potential for future drug and therapy development.

Grant:	1R21HL076526-01	
Principal Investigator:	PING, PEIPEI	PHD CARDIOVASCULAR PHYSIOLOGY
Title:	Functional Proteomic Analysis of Cardiac Mitochondria	
Institution:	UNIVERSITY OF CALIFORNIA LOS ANGELES	LOS ANGELES, CA
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): The cardiac mitochondria are critical regulators of cell survival during ischemic injury. Considerable research has been directed at interrogating molecular mechanisms that impact their normal and pathological function in the heart. Despite these efforts, to date, very limited information is known regarding the proteomic basis of this vital organelle in the normal and diseased myocardium. Consequently, a fundamental knowledge base to advance our understanding of the role of mitochondria in cardiac disease pathogenesis is lacking. At least two proteomic approaches may be utilized to map the mitochondrial sub-proteome. One focuses on broad-scale annotation of proteins (i.e., expression proteomics) and the second undertakes not only the identification but also the functional characterization of a sub-group of proteins (i.e., functional proteomics). We reason that a simple catalog of proteins without a functional correlate, or a partial catalog of proteins without information regarding the manner in which the mitochondrial proteins are organized to perform their cellular tasks, will be insufficient to generate a thorough understanding of the true proteomic basis of cardiac mitochondrial function. Thus, the application of functional proteomics is more appropriate and critically important to investigate the cardiac mitochondrial sub-proteome, because it gains proteomic information that is linked to the essential biological function of mitochondria. Recent studies have demonstrated that proteins are organized as multiprotein complexes within functional sub- 3roteomes. These multiprotein complexes are assembled to facilitate signal transduction in biological systems. Accordingly, characterization of multiprotein complexes enables not only identification of proteins in sub-proteomes but also characterization of the biological functions that they support. Nevertheless, a versatile functional proteomic Platform specifically designed to target characterization of native mitochondrial multiprotein complexes in the heart is absent. Such a platform would serve as an essential tool to delineate the proteomic basis of mitochondrial function in health and disease, and to decipher the role of multiprotein signaling complexes in this organelle. The central goal of this application is to develop a state-of-the-art proteomic platform with high-sensitivity and high-speed that is tailored for analysis of mitochondrial multiprotein complexes, to gain insights on mitochondrial protein function at a large-scale, and to explore the dynamic modulation of the mitochondrial subproteome during cardiac ischemia. The specific aims are: Aim 1--To develop and optimize a functional proteomic platform to analyze cardiac mitochondrial multiprotein complexes to discern: what proteins make up these complexes, specific protein-protein interactions among proteins within the complexes, and post-translational modifications of these proteins. Aim 2-- To elucidate the effect of myocardial ischemia on mitochondrial multiprotein complexes, protein-protein interactions, protein function, and post-translational modifications using the platform optimized in Aim 1.

Grant:	1R21HL077607-01	
Principal Investigator:	POLLARD, ANDREW E	PHD
Title:	Novel Methods for Cardiac Micro-Impedance Measurement	
Institution:	UNIVERSITY OF ALABAMA AT BIRMINGHAM	BIRMINGHAM, AL
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): Maintenance of coupling strength between cardiac myocytes is essential for normal electrical activity. That strength depends primarily on intracellular gap junction coupling and on interstitial coupling in the cleft space. Each flow path is well recognized and there is considerable evidence to suggest micro-impedance changes are important for arrhythmia initiation and maintenance. However, no procedure or standard instrument for measuring intracellular and interstitial conductivities (the inverse of impedances) has been available, so information on their magnitudes is limited. This proposal will evaluate novel theoretical and experimental approaches that, if successful, will allow intracellular, interstitial and membrane micro-impedance measurements to become a straightforward component of cardiac electrophysiologic study because no intracellular access is required. A straightforward measurement procedure would have an enormous impact on theoretical analyses, as modeling studies presently rely upon incomplete impedance data. Further development of our initial research in this Exploratory Bioengineering Research Grant (EBRG) will allow us to establish sufficient expertise to design an RO1 scale Bioengineering Research Grant (BRG) at the project's end. The project has two aims: (1) to refine the integrated theoretical and experimental approach and minimize errors in micro-impedance measurements and (2) to develop microfabricated sensor arrays for micro-impedance measurements.

Grant:	1R21GM071019-01	
Principal Investigator:	ROTH, DAVID B.	MD
Title:	Analyzing VDJ recombination at the single molecule level	
Institution:	NEW YORK UNIVERSITY SCHOOL OF MEDICINE	NEW YORK, NY
Project Period:	2004/05/01-2006/04/30	

DESCRIPTION (provided by applicant): V(D)J recombination, a site-specific DNA rearrangement reaction, is responsible for assembling immunoglobulin and T cell receptor variable region genes during lymphocyte differentiation, and it is essential for the generation of B and T lymphocytes. Aberrant V(D)J recombination events underlie a considerable fraction of lymphoid neoplasms, which are among the most common cancers. The actions of a single molecule are of utmost importance in these reactions, yet they elude the approaches available to us through "bulk biochemistry." No one has yet applied single-molecule studies to site-specific recombination; the studies we propose herein will be the first such studies conducted to understand DNA rearrangement using single-DNA micromanipulation techniques. Two specific DNA-protein complexes perform key regulatory functions in the V(D)J recombination reaction. The synaptic complex, comprising the RAG-1 and RAG-2 proteins plus accessory molecules, brings together the two DNA segments that are to undergo recombination. After DNA cleavage, the RAG proteins remain associated with the broken DNA ends in the form of a post-cleavage complex that helps direct proper joining. These two RAG-DNA complexes are critical to safeguarding the genome, yet we know little about their formation or specific activities. We propose to develop new biophysical tools to directly observe and control their formation. Specifically, we will 1) construct the necessary DNA substrates and a molecular tweezer apparatus; 2) analyze V(D)J synaptic complex formation on single tethered DNA molecules; and 3) analyze the post-cleavage complex using single-DNA studies.

Grant:	1R21HD047468-01	
Principal Investigator:	RUDOLPH, KATHERINE S	BS
Title:	Smart Knee Brace	
Institution:	UNIVERSITY OF DELAWARE	NEWARK, DE
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): A vast number of people are affected by conditions that result in profound muscle weakness or impaired motor control that impede the ability to walk. If motor function is completely lost, rehabilitation of walking function may be delayed until the patient is able move actively. Delayed therapy often leads to secondary impairments that exacerbate a patient's functional losses such as disuse atrophy and general deconditioning. Currently, therapists can begin rehabilitation of walking by using lower extremity braces or body weight supported treadmill training to support the weak limb. No currently available lower extremity braces can provide the patient the experience of typical movement patterns; rather braces are designed to immobilze the limb on which the patient bears weight. This fosters abnormal, inefficient and very energy costly movement patterns that might be difficult to overcome. During body weight supported treadmill training the body is suspended over a treadmill and all or part of the body weight is eliminated. Typical walking patterns are simulated by a manual positioning of the limb by therapists, a very time intensive and costly form of rehabilitation. The proposed project involves the development of a Smart Knee Brace (SKB) for use in rehabilitation of walking in persons with lower extremity weakness. The purpose of this study is to develop the SKB that will help to control knee movement during the stance phase of gait by encouraging more normal movement patterns while allowing free knee motion during the swing phase, something no commercially available braces can do. The SKB will provide control of the knee joint that will be adjusted for each patient based on his or her walking ability and the goals of their rehabilitation. This proposal represented the first phase of the work to develop the SBK and its electronic controllers based on walking patterns from healthy older subjects. Feasibility of the SKB will also be tested on healthy subjects and 3atients with hemiparesis from stroke. The data collected during the proposed work will be used to plan Future clinical phases of the work.

Grant:	1R21NS048248-01	
Principal Investigator:	SCHAFFER, DAVID V	PHD
Title:	Engineering Molecular Sensors for Stem Cell Function	
Institution:	UNIVERSITY OF CALIFORNIA BERKELEY	BERKELEY, CA
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): Molecular sensors are high sensitivity probes capable of reporting quantitative biological information at the molecular and cellular levels. In particular, imaging genetically encoded sensors based on fluorescent and luminescent proteins, which can be permanently introduced into cells and organisms, can yield detailed information on numerous cellular processes such as signal transduction and gene regulation. Such sensors have enormous potential for providing data to aid in the investigation of complex biological mechanisms and disease pathologies. Before their full potential can be realized, however, sensor properties must be improved, and model systems must be constructed to test and highlight their utility. Molecular sensors will be developed to study an important model system with medical potential, adult neural stem cells. This system will be employed to emphasize the idea that the complex gene regulation and signal transduction mechanisms that translate extracellular signals into intracellular decisions can be elucidated only by taking measurements at multiple junctures in these processes, in real time and at the single cell level. Firefly luciferase reporters will be developed to quantify the regulation of key transcription factors involved in two neural stem cell fate decisions, self-renewal and astrocytic differentiation, controlled by Shh and Notch signaling. Simultaneously, fluorescent protein sensors will report stem cell fate commitment. Sensor function will be validated using immunofluorescence and quantitative PCR methods. Finally, to further improve the properties of firefly luciferase reporters, a high throughput directed evolution approach based on DNA shuffling will be employed to create novel luciferase variants with higher sensitivity. Our Specific Aims are: 1) To employ molecular sensors to determine at the single cell level whether threshold levels of extracellular signals switch stem cell fate decisions 2) To determine whether higher sensitivity variants of luciferase can be created using a directed evolution approach.

Grant:	1R21EB003420-01	
Principal Investigator:	SCHMIDT, HOLGER	PHD
Title:	Integrated Optical Waveguide Sensors	
Institution:	UNIVERSITY OF CALIFORNIA SANTA CRUZ	SANTA CRUZ, CA
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): The aim of this project is the development of novel integrated optical sensors for biomedical applications. These sensors will be based on anti-resonant reflecting optical waveguides (ARROW) that allow for light propagation and guiding in low-index (liquid or gas) layers surrounded by semiconductors. Based on this principle, highly integrated instruments will be built that are compatible with fiber optic technologies and specifically avoid bulky optical setups in three dimensions involving microscopes and inefficient light coupling. This approach has several key advantages, most notably higher sensitivity, lower coupling losses and the potential for massively parallel devices due to the planar nature of the waveguide structures. Specific applications of such low-index waveguides can include fluorescence detection from single DNA molecules, highly-efficient low-volume flow cytometry, and sensitive absorption measurements of liquids containing biomolecules or gases. The implications to human health of this project range from improving fundamental understanding of DNA to more sensitive detection of potentially harmful substances in the liquid or gas phase. The specific aims of this exploratory grant are to provide the first demonstration of light guiding in low-index optical waveguides with non-solid (liquid or gas) cores and the fabrication of a first generation optical platform suitable for fluorescence measurements. The research will cover the following three areas: theory and simulation, microfabrication, and optical testing. Theoretical work will include the design of suitable ARROW waveguide structures and the calculation of detection efficiency for fluorescence in these waveguides. Microfabrication efforts will address growth and quality control of dielectric multilayer structures and fabrication of low-index channels filled with liquid or gas. Finally, prototypes will be tested with optical spectroscopy to ensure agreement with simulations and to test the robustness of the fabrication techniques. Fluorescence measurements on biological samples such as single DNA molecules will be carried out to demonstrate the potential of this integrated platform for biomedical instruments.

Grant:	1R21EB001687-01A1	
Principal Investigator:	SCHWOEBEL, PAUL R	PHD
Title:	Matrix-Addressable X-ray Source for Medical Imaging	
Institution:	SRI INTERNATIONAL	MENLO PARK, CA
Project Period:	2004/07/01-2006/06/30	

DESCRIPTION (provided by applicant): We propose to develop a new x-ray source for medical research and diagnostic applications. The development of this source could radically change the use of x-rays in areas ranging from digital tomosynthesis to computed tomography (CT). The proposed source is a high-bandwidth, matrixaddressable array of individual cold-cathode x-ray point sources that can be made to cover areas of roughly one square millimeter to several square meters. This flat panel x-ray source could be used to construct, for example, compact, flat-panel-based tomography systems for use in the field, laboratory, and rural clinic. The objective of the proposed research is to validate the operation of the flat panel x-ray source by demonstrating a functional prototype. The specific aims of this 2-year research program are to: (1) design, build, and characterize a prototype x-ray source and (2) demonstrate source operation with conventional xray radiographs and quantify source performance using standard image assessment techniques. Following the successful completion of this program we will apply for a follow-on grant to continue the refinement and development of the x-ray source. This research will involve more detailed studies of image assessment combined with further quantification of how various source preparation and operating parameters influence the x-ray panel source's output, reproducibility, and lifetime.

Grant:	1R21HL079868-01	
Principal Investigator:	SHANDAS, ROBIN	PHD
Title:	Real Time Multi-Component Blood FLow Velocimetry	
Institution:	UNIVERSITY OF COLORADO AT BOULDER	BOULDER, CO
Project Period:	2004/07/14-2006/06/30	

DESCRIPTION (provided by applicant): The development of a real-time non-invasive method to measure the multiple components of blood flow would be enormously useful for a number of cardiovascular, neurological, renal, and radiological applications. Currently, the only means of obtaining multiple blood velocity components is through MRI phase velocity mapping techniques, which are cumbersome, time-consuming, and limited in temporal resolution. Furthermore, MRI phase velocity mapping and its various counterparts are not real-time techniques. We have recently developed an ultrasound-based particle image velocimetry technique that has shown promise in the measurement of complex blood flow patterns. This method, termed echo-PIV, takes advantage of the non-linear backscatter characteristics of ultrasound contrast micro bubbles analyzed in the RF domain to distinguish individual micro bubbles, which are then tracked over time to obtain the local velocity vector. We have developed software algorithms to analyze this information and have obtained promising data from in vitro and in vivo studies. The method now requires optimization and implementation into hardware to form a real-time non-invasive velocimetry method. Our experience with ultrasound and Doppler imaging, experimental fluid dynamics including the development and use of a variety of optical PIV systems, and long-standing relationship with the ultrasound industry, make the satisfactory completion of this project highly probable. The specific aims of this project are: 1) Use numerical modeling of backscatter from micro bubbles to study which driving conditions (frequency, pulse shape, pulse length, power, etc.) will maximize the non-linear response of the bubbles and thereby increase the accuracy of the echo PIV algorithm. 2) Assemble the hardware components to implement the real-time echo PIV imaging system, based on maximizing the specifications for the two clinical applications described above. 3) Test the prototype imaging system using in vitro models with laser-based optical particle image velocimetry as the standard for comparison.

Grant:	1R21GM070600-01	
Principal Investigator:	SHMULEVICH, ILYA	PHD
Title:	Genomic Characterization - Differentiation & Homeostasis	
Institution:	UNIVERSITY OF TEXAS MD ANDERSON CAN CTR	HOUSTON, TX
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant) A cell's behavior is governed by a complex dynamical system of genetic interactions. A central role in the understanding of the nature of living systems, their stability in a changing environment, and how such systems fail in disease, such as cancer, is played by the process of differentiation. The goal of this project is to understand this process along with cellular homeostatic stability from a systems perspective. The 'state-space'of such complex nonlinear dynamical systems, representing genetic regulatory networks, consists of all possible combinations of gene activities. The regulatory interactions result in a dynamical 'flow'in this state-space. That flow or trajectory typically reaches a recurrent pattern of activities, which constitutes an attractor or the steady-state behavior of the system. Many different trajectories typically flow to the same attractor and constitute its basin of attraction. One objective of this study is to test the hypothesis that the attractors of such networks constitute the cell types of an organism, while differentiation is precisely a route (gene expression program) from one attractor into the basin of attraction of another attractor and subsequent flow to that new attractor. Another objective is to test the hypothesis that there are several distinct paths in the state-space along which cells proceed towards differentiation. A related goal is to characterize a particular differentiation process at the gene expression level. The first specific aim - mapping the molecular paths by gene expression profiling for differentiation pathways - is intended to achieve these objectives. Finally, another objective is to study the process of cellular homeostasis on the gene expression level. The particular questions related to this objective are: do the cells exhibit homeostasis on the expression level by returning to their original states in the state-space and if so, do they retrace the same trajectory on their way back? Thus, the second specific aim - the study of homeostatic stability on the gene expression level - is proposed to realize this objective. The methods designed to achieve these goals include treating HL60 promyelocytic leukemia cells, a well-established differentiation model, with different doses and durations of all-trans retinoic acid (ATRA) and dimethyl sulfoxide (DMSO), to differentiate the cells into monocytes and granulocytes, respectively. Using early differentiation cell surface markers (CD11b) and flow cytometry, the investigators will construct loci on the dose-duration plane such that a given locus corresponds to a fixed percentage of differentiated cells. Given several different treatments that place the cells on the same locus, the cells will be profiled at different time points with microarrays in order to determine whether they follow distinct paths of differentiation. With additional microarray profiling of untreated cells, gene sets important for monocytic and granulocytic differentiation on different loci will be revealed. In order to study homeostatic stability, cells will be treated such that they are on the 50% locus and microarray profiling will be performed at different time points during treatment. After live sorting of the cells using CD11b, the CD11b positive and negative cells will be cultured in the absence of differentiation inducing agents. Microarrays will be used to profile each of these cell populations using time-point measurements, thus making possible the characterization of homeostatic behavior on the gene expression level.

Grant:	1R21EB004066-01	
Principal Investigator:	SIMPSON, MICHAEL L	PHD
Title:	Nanostructure Gene Delivery Arrays	
Institution:	UNIVERSITY OF TENNESSEE KNOXVILLE	KNOXVILLE, TN
Project Period:	2004/08/01-2006/07/31	

DESCRIPTION (provided by applicant): The focus of this research will be to evaluate vertically-aligned carbon nanofiber (VACNF) arrays as a parallel interface to deliver macromolecules, specifically different DNAs, in a spatially resolved manner to cellular matrices. Our nanofiber-based approach exploits a universal delivery mechanism, microinjection, that has been demonstrated as effective for all cell types (mammalian, plant, bacterial). However, unlike conventional microinjection, which is a labor intensive and slow serial method for single cell delivery, vast arrays of VACNFs can provide for massively parallel gene delivery to cellular matrices. Spatially resolved delivery will allow the production of arrays of genetically manipulated live cells that can be used as a powerful discovery tool. Examples of applications and future R01 efforts that can be supported by successful demonstration of this tool include the real-time functional characterization of large ensembles of expressed gene products, the evaluation of specificity of candidate drugs on libraries of over-expressing transformed cells, and loss-of-function analyses in cell groups receiving or producing interfering RNAs. Towards this goal, this effort will evaluate the potential of nanofiber-mediated gene delivery, with the specific target of spatially resolved material delivery to cellular matrices for live-cell arraying. In this R21 effort we will: 1. Investigate strategies for temporarily and permanently immobilizing different DNAs in discrete patterns to localized regions of periodic arrays of vertically-aligned carbon nanofibers grown on planar substrates 2. Incorporate these DNA-modified arrays into cellular matrices in a parallel 'microinjection'-based scheme and subsequently quantify the effectiveness of plasmid delivery and expression within targeted cells. 3. Initially evaluate nanofiber-mediated methods for live cell microarraying of diverse cell types; including both mammalian and yeast cells.

Grant:	1R21EB003869-01	
Principal Investigator:	SONG, YI-QIAO	PHD
Title:	A new MR method to determine bone strength	
Institution:	SCHLUMBERGER-DOLL RESEARCH	RIDGEFIELD, CT
Project Period:	2004/09/01-2006/08/30	

DESCRIPTION (provided by applicant): Osteoporosis is a disorder of the skeleton in which bone strength is abnormally weak and susceptible to fractures from minor trauma. In the United States, about 30 million people have osteoporosis and almost 19 million more have low bone density. Current diagnostics of osteoporosis using bone density does not entirely predict fracture risk, because the internal bone structure, apart from the bone density, contributes significantly to the mechanical strength and thus fracture risk. Recent efforts have been directed toward the high-resolution three-dimensional imaging of the trabecular architecture using Magnetic Resonance Imaging (MRI) and Computed Tomography. The main MR technique (DDIF) used in this proposal is a completely new concept in bone characterization. This study proposes to use the DDIF technique to obtain statistical properties of the trabecular structure at a resolution of about 1 mu m, such as a pore size distribution, instead of high-resolution images. Preliminary work has obtained pore size distributions from bone samples and these distributions have shown a direct correlation with the mechanical properties. The long-term objective of this research is the development of new DDIF-based MR methods for in vivo evaluation of bone strength. Specific aims of the proposed research are: (1) To establish the correlation of DDIF pore size distribution with the mechanical properties of bone, (2) To develop pulse sequences to combine DDIF with MR imaging and spatial localization techniques and to quantify the accuracy of DDIF through numerical analysis. This proposal is designed to develop specific methods to incorporate DDIF into clinical MRI systems and to provide a solid evidence for the new paradigm of bone evaluation. This development will enable the next phase of the project to perform in vivo study of DDIF to further validate the method for clinical osteoporosis diagnostics and the treatment monitoring.

Grant:	1R21GM070826-01	
Principal Investigator:	STEWART, RUSSELL J	PHD
Title:	Self-immobilizing Proteins	
Institution:	UNIVERSITY OF UTAH	SALT LAKE CITY, UT
Project Period:	2004/06/18-2006/05/31	

In nature, highly crosslinked networks of proteins and other biopolymers are common materials. In many cases, dityrosine crosslinks form spontaneously after tyrosine sidechains are enzymatically oxidized into reactive intermediates within a restricted region. The overall objective of the proposed research is to develop a novel site-specific protein immobilization chemistry similar to natural dityrosine crosslinking mechanisms. Crosslinks between strategicallly placed phenolic groups (e.g., tyrosine) will be catalyzed in the presence of a mild oxidant by a metal complex between a synthetic metal ligand and a metal binding peptide genetically appended to the protein. Specifically, this objective will be pursued by: i.) solid-state synthesis of combinatorial libraries of peptidic metal binding ligands, ii.) rapid on-bead library screening with labelled tyrosine-containing model peptides to discover a ternary metal complex that catalyzes dityrosine formation, iii.) optimization of catalytically active leads through refined searching within the positive parameter space, and iv.) testing of active complexes with model proteins to demonstrate the utility of self-immobilizing proteins. The proposed chemistry may have major advantages over existing protein modification methodologies. First, the proposed method does not rely on diffusible reagents that react with all accessible members of a particular class of nucleophilic functional group. Rather, protein modification will be localized to specific sites determined by the pre-formation of a ternary metal complex. Second, the protein modification site is determined genetically, eliminating the need for post-translational modification and allowing specific protein immobilization from complex mixtures. An important health related application of the proposed technology will be more efficient immobilization of proteins into arrays on solid supports. The widely expected future impact of protein arrays on clinical diagnosis and other areas of human health care may be realized more quickly with new and effective protein modification technology.

Grant:	1R21EB003951-01	
Principal Investigator:	WEHRLI, FELIX W	
Title:	NMR Q-Space imaging of tissue microarchitecture	
Institution:	UNIVERSITY OF PENNSYLVANIA	PHILADELPHIA, PA
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): MRI has proven its potential for noninvasive assessment of tissue architecture. However, the achievable spatial resolution is ultimately determined by the modality's limited detection sensitivity in terms of signal-to-noise ratio (SNR). An alternative approach toward characterizing the microarchitecture of structured materials and tissues is q-space imaging. Analogous to k-space and image space representing spatial frequency and its Fourier transform -- the spectrum of spin density -- the Fourier transform of the NMR q-space signal is the spectrum of displacements. In the white matter of the spinal cord an array of axons provides a quasi-regular array of parallel cylindrical structures separated from the extracellular medium by a semi-permeable myelin sheaths which imposes barriers to diffusion. Q-space NMR currently has several limitations. The first is the amplitude of the diffusion sensitizing gradients, since qmax determines the resolution in the displacement domain. For example, for a propagator resolution of 1 mu/m and a gradient duration delta=1ms Gmax= 2,200 G/cm would be required, which is more than one order of magnitude greater than gradient strengths typically available. Access to such gradient capabilities further will allow probing of very small-scale diffusion restrictions. Second, while simulations of the q-space behavior have the potential to provide physical and biological insight and enable systematic planning of experiments, such approaches demand more elaborate models than those hitherto available. The investigators have, in preliminary work, explored the above issues and propose to further develop and apply q-space methodology for the non-destructive analysis of tissue microstructure and function, focusing on the axonal structure of the rat spinal cord. The overall hypothesis underlying this proposal is that ultra high-resolution displacement imaging in conjunction with simulations of diffusion diffraction from histologic images will provide new insight into tissue architecture. The following specific aims will be pursued: 1. Complete construction and evaluate the performance of a single-axis gradient system allowing amplitudes of up to 4,000 G/cm for performing q-space imaging of small specimens at 400 MHz. 2. Simulate q-space behavior on the basis of a previously developed finite difference model for (a) synthetic models of axons as a function of distribution of axon size and membrane permeability, (b) histologic images of rat spinal cord for different white matter regions. 3. Perform high-q 3D q-space imaging in excised rat spinal cord with the objective of quantifying axonal architecture and compare the results with histology.

Grant:	1R21AR051564-01	
Principal Investigator:	WREN, TISHYA AL	PHD
Title:	Mechanical Intervention in Children with Cerebral Palsy	
Institution:	CHILDREN'S HOSPITAL LOS ANGELES	LOS ANGELES, CA
Project Period:	2004/09/01-2006/08/31	

DESCRIPTION (provided by applicant): Peak bone mass, which is achieved soon after the end of sexual development, is the most important determinant of bone mass and osteoporosis later in life. Disabled children, such as children with cerebral palsy, are particularly vulnerable to deficits in bone mass accretion due to decreased mobility and weight-bearing which reduces mechanical loading of the skeleton. In addition, these children have poor muscle strength and function which contributes to the lack of mechanical stimulation needed to build bone mass. Physical therapy can increase bone mass and improve muscle function, but the therapy is time and labor intensive and may not be available as frequently as needed. Whole body vibration has shown promise as an alternative method for stimulating both increases in bone mass and improvements in muscle performance. In whole body vibration interventions, the subject stands on a platform that vibrates either up and down or in a rocking motion. The main purpose of this proposal is to test the efficacy of high frequency, low magnitude vibration as an intervention for low bone mass and poor muscle function in children with cerebral palsy. Specifically, this study will test the following hypotheses: (1) The vibration intervention will increase axial and appendicular bone density and long bone cross-sectional properties relative to controls; (2) The vibration intervention will increase muscle mass, muscle strength, and postural stability relative to controls; (3) The vibration intervention will decrease markers of bone resorption and increase markers of bone formation relative to controls. In the vibration intervention, subjects will receive mechanical stimulation for 10 rain/day, 7 days/wk for 6 months. These subjects will undergo an additional 6 months of weight bearing without vibration following the same schedule to serve as an internal control. An additional control group of matched children will receive no intervention. Changes in lumbar spine and proximal tibia cancellous bone density, cross-sectional properties of the midshaft of the tibia, markers of bone turnover, calf muscle cross-sectional area and strength, and indices of postural stability will be measured to determine whether or not the vibration intervention is effective in improving bone mass and muscle function. Using CP as a model, this study will help to validate or invalidate the general usefulness of low magnitude mechanical stimulation as an intervention for osteoporosis. This study will also provide insight into the potential role of muscle adaptation and bone turnover in increasing bone mass in response to this simple, non-invasive, non-pharmacalogical intervention.

Grant:	1R21EB003602-01A1	
Principal Investigator:	YANG, VICTOR C	
Title:	Brain Drug Delivery Using Parkinson as a Disease Model	
Institution:	UNIVERSITY OF MICHIGAN AT ANN ARBOR	ANN ARBOR, MI
Project Period:	2004/09/24-2006/08/31	

DESCRIPTION (provided by applicant): The protection by the bone-structured skull, the lack of targeting or retention specificity, and the presence of the impermeable blood brain barrier (BBB) render the brain the least promising territory for drug intervention. In this R21 application, we propose an innovative brain drug delivery approach by utilizing the biodegradable clinical MR imaging agent, the superpara-magnetic iron oxide nanoparticles (MION), as the drug carrier and a clinical magnetic field as the tool for overcoming the skull barrier thereby achieving the specific brain-site targeting. Transferrin ligands will be immobilized onto the dextran coating of the MION particles for localization and retention of the targeted MION onto brain capillaries. TAT, a potent cell transduction peptide derived from the HIV protein, will also be linked to the dextran coating to serve as the contrivance for overriding the BBB and cell membrane barriers. It has been demonstrated in animal studies that via covalent linkage, TAT was able to transduce MION into organ tissues including the brain. To attenuate the non-specific uptake of MION by normal tissues, the trans-membrane activity of TAT will be masked via the binding with heparin. It has been confirmed that heparin can completely inhibit TAT-mediated cell transduction in vivo. A pharmacokinetic study will be conducted to determine the time required for MION to reach the maximum localization at the brain site but minimum systemic distribution. Protamine, a clinical heparin antidote, will be administered at this pre-determined time frame to dissociate heparin from its electrostatic binding to TAT. Once relieved from heparin inhibition, TAT will resume its potent trans-membrane activity, enabling MION to cross BBB and enter brain cells. Inside the brain, drug molecules, which will be linked to the dextran coating via hydrolysable bonds, will be slowly released from MION, sustaining a therapeutic concentration of the drug over an extended period of time. Parkinson's disease (PD) will be selected as the disease model to assess the feasibility of this approach in delivering dopamine into the brain. This is primarily because that PD offers a sensitive and clinically relevant animal model (i.e. the 6-OHDA rat model) that produces both physical (e.g. kinesic) and chemical (e.g. TH immunohistochemistry) responses in a direct correlation to the brain dopamine concentration and activity. Therefore, the success or failure of this brain drug delivery approach can be unquestionably confirmed from the experimental results. Since the 6-OHDA rat PD model can be applied in a reverse manner to examine the neuroprotective effects of peroxidase, a potent H202 scavenger that can protect neurons from attack by free radicals, delivery of peroxidase will also be attempted to see if this can retard PD progression. Because of the restricted budget and short duration of the R21 grant, this application plans to take a shotgun approach to achieve the proof-of-concept of this project, by conducting primarily in vivo animal studies. However, if the approach proves feasible in delivering both the hydrophilic dopamine and large peroxidase protein (two drugs that cannot cross BBB), a greatly extended R01 application basing on brain delivery of neurotrophic factors for promoting neuronal survival, stimulating axonal growth, and altering the course of the underlying 9 disease, will be followed to achieve the ultimate PD treatment.

Grant:	1R21EY015457-01	
Principal Investigator:	YIU, SAMUEL C	PHD
Title:	Bioartificial Lacrimal Gland	
Institution:	DOHENY EYE INSTITUTE	LOS ANGELES, CA
Project Period:	2004/05/01-2006/04/30	

DESCRIPTION (provided by applicant) Millions of Americans have dry eye disease. Individuals plagued by the discomfort, burning, irritation, photophobia, and other symptoms of dry eye disease also have blurred vision, contact lens intolerance, the inability to produce emotional tears, and an increased risk of ocular surface damage and infection. In the United States alone an estimated 2 million Sjogren's Syndrome patients have dysfunctional lacrimal glands and severe dry eye and there is no satisfactory treatment. A bioartificial lacrimal gland would greatly benefit these patients. The new field of tissue engineering has built on the interface between materials science and biocompatibility to create the possibility of developing a bioartificial lacrimal gland. Our three specific aims are: 1) To identify the optimal biomaterials for use as the substrate for the growth of rabbit lacrimal epithelial cells in a three-dimensional scaffold. 2) To test the physiological properties of these bioengineered tissues, including secretory functions and electrophysiological activities. 3) To establish strategies and data for the design and development of a bioartifical lacrimal gland as the basis for a BRG grant proposal using the RO1 funding mechanism. This work will advance our understanding of how lacrimal epithelial cells function in this artificial environment and lead to the development of an RO1 proposal. Ultimately, we envision a bioengineered lacrimal gland system, to be surgically implanted in periocular tissues that will produce sufficient tear flow to maintain the health of the ocular surface. Such a device could relieve the symptoms of millions of dry eye patients; and it could possibly make obsolete the frequent daily use of lubricant eye drops, saving patients the time and effort of medication use and saving millions of dollars annually in the purchase of lubricating eye drops.

Grant:	1R21DE016370-01	
Principal Investigator:	YOUNG, CONAN	PHD
Title:	Coordinated bioengineering of tooth and bone in the jaw	
Institution:	FORSYTH INSTITUTE	BOSTON, MA
Project Period:	2004/09/28-2006/07/31	

DESCRIPTION (provided by applicant): Tooth loss continues to plague many Americans, and is complicated by bone resorption in the jaw. Current therapies for tooth replacement include synthetic dental implants, but these devices do not maintain the health of surrounding tissues and require sufficient amounts of alveolar bone to secure the implant. The ability to replace lost or diseased dentition and alveolar bone with healthy, biological teeth would revolutionize the practice of dentistry and oral surgery. Our long-term goal is to bioengineer biological tooth replacements containing enamel, dentin and pulp, and roots anchored to the underlying alveolar bone via periodontal ligament. Our current bioengineered tooth Crowns exhibit variable amounts of enamel and Jentin, and lack mature root structures. We expect that bioengineered tooth root formation will be enhanced by growth in close proximity to developing bone. This hypothesis will be tested by growing composite bioengineered tooth/bone constructs in the jaws of Yucatan mini-pigs. Excised implants will be analyzed for the presence of tooth crowns, mature tooth root structures, and alveolar bone. We will empirically determine the specific ratio of dental epithelial to mesenchymal cells seeded onto scaffolds required to bioengineer dentin and enamel of appropriate relative thickness. Thus, our Specific Aims are to: 1) demonstrate the coordinated formation of tooth crowns, roots and bone by bioengineering tooth and alveolar bone segments grown in the jaws of pigs, and 2) to bioengineer tooth crowns containing the relative amounts of enamel and dentin present in naturally formed teeth. We anticipate that the successful bioengineering of mature teeth anchored to alveolar bone via roots and periodontal ligament will provide an alternative therapeutic treatment for dental and oral cancer patients, and for individuals suffering from a variety of craniofacial defects.

Grant:	1R21HL076356-01	
Principal Investigator:	YU, HONG	PHD
Title:	Bioengineering of a Stent-Graft for Gene Therapy	
Institution:	UNIVERSITY OF MIAMI-MEDICAL	CORAL GABLES, FL
Project Period:	2004/04/01-2006/03/31	

DESCRIPTION (provided by applicant): A stent-graft is a conduit composed of a polymer membrane supported by a metal stent that is placed in a vessel using catheter technology. We have genetically engineered vascular smooth muscle cells (SMC) and placed them on a stent graft that was specifically designed to shelter the SMC cells from implantation trauma. We found that these genetically engineered cells survived and proliferated and that gene expression was maintained at high levels over a long period, indicating the feasibility of this new gene therapy strategy to deliver the gene product directly into the bloodstream. The objective of this project is to explore whether a stent-graft suffused with genetically engineered SMC can be used to deliver functional Factor IX (F.IX) to treat hemophilia B. Hemophilia B is an X-linked bleeding diathesis resulting from a deficiency of blood coagulation factor IX. Hemophilia is an ideal model for gene therapy because precise regulation and tissue-specific transgene expression are not required. We will use a hemophilic dog model to study the feasibility of bioengineering a stent graft for gene therapy. We hypothesize that the intravascular delivery of F. IX using a stent-graft suffused with retrovirally transduced SMC will offer the opportunity for delivery of F.IX at a therapeutic level to correct the coagulation defect. We will first determine how long and how much of the transgene product canine F.IX can be produced from the bioengineered stent grafts after being implanted into the aorta of a hemophilia B dog using catheter technology. We will modulate the level of F.IX production by the length of the implanted stent graft. Then, we will determine whether the secreted F. X at these levels can ameliorate the coagulation defect in a hemophilic dog by measuring coagulation parameters. The host immune response to the transgene product canine F.IX will also be examined. The outcome of the project will have direct applications in the treatment of hemophilia as well as other blood and vascular disorders.